IS 3839 (1989): Food Yeast [FAD 16: Foodgrains, Starches and Ready to Eat Foods]

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IS 3839 : 1989
(Reaffirmed 2005)

Indian Standard

FOOD YEAST — SPECIFICATION

(First Revision)

भारतीय मानक

खाद्य खमीर — विशिष्टि

(पहला पुनरीक्षण)

UDC 664·872

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Price Group 4

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards on 2 June 1989, after the draft finalized by the Bakery and Confectionery Industry Sectional Committee had been approved by the Food and Agriculture Division Council.

Food yeast is produced from surplus sugarcane extracts, sugars from wood-pulp waste, waste sulphite liquors and molasses on which yeast suitable for human consumption belonging to the genera *Saccharomyces* and *Torula* is grown. Though there are variations in details of the manufacturing processes, the production of food yeast basically involves:

a) Selection of suitable strains of yeast;

b) Propagation of selected yeast to build up sufficient seed yeast;

c) Preparation of nutrient medium;

d) Separation of yeast in suspension and washing it to get rid of salts, gums, colouring matter, unpleasant flavours and residual liquor surrounding the cells; and

e) Filtration and suitably drying to fine powder or flakes.

The yeast may also be autolysed and flavoured before drying.

Food yeast is a rich source of protein and B-complex vitamins. It is, therefore, incorporated in food products, such as, bread, biscuits, baby foods, canned meats, gravies, soups, puddings, candy and ice creams to enhance their nutritive value and to impart the flavour.

This standard was first published in 1966. This revision incorporates Amendments No. 1 and 2. In addition, the limit for microflora other than yeast has been reduced and mould count has been incorporated.

While formulating this standard, due consideration has been given to the relevant Rules framed by the Government of India under the *Prevention of Food Adulteration Act, 1954*. This standard is subject to the restriction imposed under that Act, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

FOOD YEAST — SPECIFICATION

(First Revision)

1 SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for food yeast.

2 REFERENCES

2.1 The following Indian Standards are necessary adjuncts to this standard:

IS No.	*Title*
IS 1320 : 1988	Specification for baker's yeast (*third revision*)
IS 2491 : 1972	Code for hygienic conditions for food processing units (*first revision*)
IS 5398 : 1969	Methods for estimation of thiamine (*Vitamin* B_1) in foodstuffs
IS 5399 : 1969	Methods for estimation of riboflavin (*Vitamin* B_2) in foodstuffs
IS 5400 : 1969	Methods of estimation of nicotinic acid (niacin) in foodstuffs
IS 5403 : 1969	Method for yeast and mould count in foodstuffs
IS 7219 : 1973	Method for determination of protein in foods and feeds

3 TYPES

3.1 The material shall be of two types as given below:

a) Food yeast, and

b) Autolysed yeast.

The food yeast shall be made from yeast belonging to the genus *Torula* and *Saccharomyces* and autolysed yeast from that belonging to the genus *Saccharomyces*.

4 REQUIREMENTS

4.1 The material shall be in the form of powder or flakes It shall be of uniform creamy white to dark yellow colour and shall have the characteristic taste and odour of good quality yeast, free from any unpleasant, musty or putrid smell. It shall be dry and free from lumps, rodent contamination, visible mould growth and insect infestation. It shall also be free from extraneous matter, added colour and deleterious substances.

NOTE — The appearance, taste and odour of yeast shall be determined by organoleptic test.

4.2 The material shall be manufactured in the premises maintained under hygienic conditions specified in IS 2491 : 1972.

4.3 The material shall also comply with the requirements given in Table 1.

Table 1 Other Requirements for Food Yeast

(*Clauses* 4.3, 8.1 *and* F-3.4.1)

Sl No.	Characteristic	Requirement		Method of Test	
		Food Yeast	Autolysed Yeast	Ref to Annex to This Standard	Other Indian Standards
(1)	(2)	(3)	(4)	(5)	(6)
i)	Moisture, percent by mass, *Max*	8·0	4·5	—	Appendix A of IS 1320 : 1988
ii)	Total ash (on dry basis), percent by mass, *Max*	8.5	10·0	A	—
iii)	Acid-insoluble ash (on dry basis), percent by mass, *Max*	0·05	0·05	B	—
iv)	Crude protein (N×6·25) (on dry basis), percent by mass, *Max*	50·0	30·0	—	IS 7219 : 1973
v)	Amino nitrogen, mg/100 g, *Min*	350·0	150·0	C	—
vi)	Thiamine, mg/100 g, *Min*	10·0	10·0	—	IS 5398 : 1969
vii)	Riboflavin, mg/100 g, *Min*	10·0	10·0	—	IS 5399 : 1968
viii)	Nicotinic acid, mg/100 g, *Min*	50·0	50·0	—	IS 5400 : 1969
ix)	Microflora other than yeast (on dry mass basis), *Max*	50 000/g	50 000/g	—	Appendix E of IS 1320 : 1988
x)	Mould count (on dry mass basis), *Max*	100/g	100/g	—	IS 5403 : 1969
xi)	Dispersibility in water	To pass test	To pass test	—	Appendix B of IS 1320 : 1988
xii)	Viability	To pass test	To pass test	D	—
xiii)	Copper, mg/kg, *Max*	60	60	E	—
xiv)	Lead, mg/kg	5	5	F	—

5 PACKING

5.1 The material shall be packed in clean, sound and suitable containers in such a manner as to prevent absorption of extraneous moisture and undue deterioration during storage.

6 MARKING

6.1 Each container shall be suitably marked to give the following particulars:

a) Name of the material and trade-mark, if any;
b) Name and address of the manufacturer;
c) Date of manufacture;
d) Batch or code number;
e) Net weight in g or kg; and
f) Any other details required under the *Standards of Weight and Measures (Packaged Commodities), Rules, 1977/PFA Rules.*

7 SAMPLING

7.1 Representative samples of the material shall be drawn and the criteria for ascertaining conformity of the material to the requirements of this specification shall be as prescribed in Annex G.

8 TESTS

8.1 Tests shall be carried out as prescribed in **4.1** and col 5 of Table 1.

8.2 Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS 1070 : 1977) shall be used where the use of water as reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A
[*Table* 1, *Item* (ii)]
DETERMINATION OF TOTAL ASH

A-1 PROCEDURE

A-1.1 Weigh accurately about 10 g of the material in a tared porcelain, silica or platinum dish. Ignite with the flame of a suitable burner for about one hour. Complete the ignition by keeping in a muffle furnace at 600 ± 20°C until grey ash results. Cool in a desiccator and weigh. Ignite the dish again in the muffle furnace for 30 minutes, cool and weigh. Repeat this process of igniting in a muffle furnace, cooling and weighing till the difference in mass between the two successive weighings is less than one mg. Note the lowest mass.

A-2 CALCULATION

A-2.1 Total ash (on dry basis),

$$\text{percent by mass} = \frac{10\,000\,(M_2 - M)}{(100 - X)(M_1 - M)}$$

where

M_2 = lowest mass in g of the dish with ash,
M = mass in g of empty dish,
X = moisture, percent by mass, and
M_1 = mass in g of the dish with the material taken for the test.

ANNEX B
[*Table* 1, *Item* (iii)]
DETERMINATION OF ACID INSOLUBLE ASH

B-1 REAGENT

B-1.1 Dilute Hydrochloric Acid, approximately 5 N, prepared from concentrated hydrochloric acid.

B-2 PROCEDURE

B-2.1 Obtain the total ash by igniting the dried material, after the determination of moisture, with the flame of a suitable burner and complete the ignition by keeping in a muffle furnace at 600 ± 20°C until grey ash results. Add 25 ml of dilute hydrochloric acid, cover with a watch glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from acid and transfer the filter paper to the dish. Keep the dish in an air-oven maintained at 105 ± 2°C for about 3 hours. Ignite in a muffle furnace at 600 ± 20°C for one hour. Cool the dish in a desiccator and weigh. Heat again at 600 ± 20°C in the muffle furnace for 30 minutes. Cool the dish in the desiccator and weigh. Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighings is less than one mg. Note the lowest mass.

B-3 CALCULATION

B-3.1 Acid-insoluble ash (on dry basis),

$$\text{percent by mass} = \frac{10\,000\,(M_2 - M)}{(100 - X)(M_1 - M)}$$

where

M_2 = mass in g of the dish with the acid-insoluble ash,
M = mass in g of empty dish,
X = moisture, percent by mass, and
M_1 = mass in g of the dish with the material taken for the test

ANNEX C

[*Table* 1, *Item* (v)]

DETERMINATION OF FREE AMINO NITROGEN

C-1 REAGENTS

C-1.1 Phenolphthalein Indicator Solution

C-1.2 Barium Hydroxide Solution, 0·2 N.

C-1.3 Hydrochloric Acid, 0.2 N.

C-2 PROCEDURE

C-2.1 Weigh accurately about 2·5 g of the material in 250 ml Erlenmeyer flask. Add 100 ml of water, shake thoroughly and let stand for one hour shaking every 10 to 15 minutes. Centrifuge the contents and filter, if necessary. Collect the aliquot in a 100-ml volumetric flask and make up the volume.

C-2.2 Pipette 20 ml of the aliquot into a conical flask and neutralize it to phenolphthalein, using barium hydroxide solution. Titrate the contents with 0·2 N barium hydroxide solution until distinct red colour appears. Add small but known excess of 0·2 N barium hydroxide and back titrate to neutrality with 0·2 N hydrochloric acid. Note the volume of barium hydroxide.

C-2.3 Carry out blank titration with the same reagents using 20 ml of water in place of the aliquot and note the volume of barium hydroxide.

C-3 CALCULATION

C-3.1 Amino nitrogen, mg/100 g $= \frac{1\,400\,(A-B)}{M}$

where

A = volume of barium hydroxide in sample titration,

B = volume of barium hydroxide in blank titration, and

M = mass in g of the material taken for the test.

ANNEX D

[*Table* 1, *Item* (xii)]

DETERMINATION OF VIABILITY

D-1 REAGENT

D-1.1 Yeast Nutrient Mixture

Grind and mix thoroughly 4 g of sucrose, 0·50 g of di-ammonium phosphate and 0·25 g of magnesium sulphate.

D-2 PROCEDURE

D-2.1 Add 4.5 g of yeast-nutrient mixture to a 100-ml pasteur flask containing 50 ml of tap water and sterilize by heating in an autoclave for 20 minutes at 115°C; add with aseptic precautions 2 g sample and incubate at 30°C. Collect the gas evolved into a graduated tube inverted over the outlet tube in a trough of water and measure the volume of gas at the end of 6 hours. Repeat the operation omitting the sample and subtract the volume of gas evolved from that obtained in the first determination. The sample shall be considered to have passed the test if the difference is not greater than 10 ml.

ANNEX E

[*Table* 1, *Item* (xiii)]

DETERMINATION OF COPPER

E-1 METHODS

E-1.1 Two methods, namely the spectrophotometric (*see* **E-2**) and the gravimetric (*see* **E-3**). are prescribed for determination of copper in the material. Whereas the spectrophotometric method should be used for referee purposes, the gravimetric method may be used for routine analysis wherever facilities or spectrophotometric analysis are not available.

E-2 SPECTROPHOTOMETRIC METHOD

E-2.1 Apparatus

E-2.1.1 *Spectrophotometer,* of a suitable type.

E-2.2 Reagents

E-2.2.1 *Concentrated Sulphuric Acid,* sp gr 1·84.

E-2.2.2 *Sodium Carbonate,* solid.

E-2.2.3 *Concentrated Hydrochloric Acid,* sp gr 1.16. diluted with an equal volume of water.

E-2.2.4 *Citric Acid,* solid.

E-2.2.5 *Ammonium Hydroxide Solution,* sp gr 0.90.

E-2.2.6 *Sodium Diethyldithiocarbamate Solution,* 0·1 percent (*m*/*v*), aqueous.

E-2.2.7 *Carbon Tetrachloride,* redistilled.

E-2.2.8 *Sodium Sulphate,* anhydrous.

E-2.2.9 *Concentrated Nitric Acid,* sp gr 1·42, diluted with an equal volume of water.

E-2.2.10 *Standard Copper Solution*

Weigh accurately 0·100←0←g of pure copper turnings, carefully dissolve in minimum amount of nitric acid, cool and dilute to one litre. Pipette 10 ml of this solution into a 100-ml volumetric flask and dilute to the mark. This solution contains 10 μg of copper per ml.

E-2.3 Procedure

E-2.3.1 *Preparation of the Test Solution*

Weigh accurately about 20·0 g of the material in a platinum dish and add to it 2 ml of concentrated sulphuric acid. Heat the dish gently over a Bunsen burner until charring is complete and ash the residue in a muffle furnace at 550 to 600°C. Cool the dish, add to the ash about 2 g of sodium carbonate and fuse the contents of the dish for 10 minutes at 900°C. Cool the dish and dissolve the fused matter in the minimum amount of hydrochloric acid, covering the dish with a watch-glass to avoid loss by spattering. Heat the dish until the solution is complete. Cool the dish and make up the solution to 100 ml in a volumetric flask with water.

E-2.3.2 Transfer 10 ml of the test solution to a separating funnel by means of a pipette. Add 1 g of citric acid to the test solution and dissolve it by shaking. Make the resulting solution alkaline to litmus paper by adding ammonium hydroxide solution in small quantities. Add to this alkaline solution, 5 ml of sodium diethyldithiocarbamate solution, shake thoroughly and extract with 5-ml portions of carbon tetrachloride until the final extract is colourless (about four extractions are usually adequate). Dry the combined extracts by shaking thoroughly with anhydrous sodium sulphate. Filter the dry extract and wash the filter paper with carbon tetrachloride. Make up the volume of the filtrate to 25 ml with carbon tetrachloride and measure the absorption at 437 nm by means of the spectrophotometer.

E-2.3.3 Prepare a series of standard colour solutions, using different volumes of standard copper solution instead of 10 ml of the test solution and proceeding as described under **E 2.3.2**. This series should cover the concentration of the colour solution prepared from the test solution. Measure the absorption of each of the colour solutions in the series.

E-2.3.4 Carry out blank determinations on water and the reagents used in the preparation of the standard colour solutions (*see* **E-2.3.3**) and the colour solution from the material (*see* **E-2.3.1** and **E-2.3.2**). If the values so obtained are of any significance, correct the respective values observed for the series of standard colour solution (*see* **E-2.3.3**) and the test solution (*see* **E-2.3.2**) accordingly.

E-2.3.5 Plot a curve using the corrected values for absorption and the corresponding concentration of copper in micrograms present in standard colour solution in the series. From this curve, obtain the weight in μg of copper present in 10 ml of the test solution (*see* **E-2.3.1**).

E-2.4 Calculation

$$\text{Copper content of the material, mg/kg} = \frac{10\ M}{W}$$

where

M = mass, in μg, of copper present in 10 ml of the test solution (*see* **E-2.3.5**); and

W = mass in g of the material taken for the test.

E-3 GRAVIMETRIC METHOD

E-3.1 Reagents

The following reagents are required. The reagents shall be free from traces of copper.

E-3.1.1 *Concentrated Sulphuric Acid,* sp gr 1·84.

E-3.1.2 *Sodium Carbonate,* solid.

E-3.1.3 *Concentrated Hydrochloric Acid,* sp gr 1·16, diluted with an equal volume of water.

E-3.1.4 *Sodium Hydroxide Solution,* approximately 2 N.

E-3.1.5 *Sodium Acetate,* crystalline.

E-3.1.6 *Glacial Acetic Acid*

E-3.1.7 *Salicylaldoxime Solution*

Dissolve 1 g of salicylaldoxime in 5 ml of rectified spirit without heating. Gently pour this solution into 95 ml of water at 80°C. The oxime partially separates out in the form of a fine oil suspension, but quickly redissolves. Avoid shaking at this stage as shaking helps the small droplets of the oxime grow. When the solution becomes clear, shake it for some time and filter. Use the filtrate.

E-3.1.8 *Ferrous Chloride Solution,* about 5 percent (*m/v*).

E-3.2 Procedure

E-3.2.1 Proceed as described under **E-2.3.1** but do not make up the volume to 100 ml.

E-3.2.2 To the whole of the solution so obtained, add sodium hydroxide solution until a lasting precipitate is formed. Add 1 g of sodium acetate and 10 ml of glacial acetic acid, and stir until the precipitate re-dissolves. Dilute the resulting, solution to about 100 ml with water. Add to this dilute solution a bare excess of salicylaldoxime solution to precipitate all the copper present in the solution. Coagulate the precipitate by stirring with a glass rod and allow to settle. Test the supernatant liquid for the completion of precipitation by adding several drops of salicylaldoxime solution. (A considerable excess of salicylaldoxime should be avoided as the precipitate has to be washed free from it before drying; otherwise, the precipitate would be visibly decomposed during drying.) Filter the precipitate through a tared No. 3 Gooch crucible and wash it with cold water until the filtrate ceases to give any colour with ferrous chloride solution. During washing, take care that the precipitate always remains moist. Finally wash the precipitate twice more with water and dry it, as far as possible, by suction. Dry the

crucible with contents to constant mass at 105 to 110°C, cool in a desiccator and weigh. Find the mass of the dried precipitate.

E-3.3 Calculation

$$\text{Copper content of the material, mg/kg} = \frac{1\,89\,200\,m}{M}$$

where

m = mass, in g, of dried precipitate as determined under **E-3.2.2**; and

M = mass, in g of the material taken for the test.

ANNEX F

[*Table* 1, *Item* (xiv)]

DETERMINATION OF LEAD

F-1 PRINCIPLE

F-1.1 Using the residue obtained under **F-3.1**, the lead content is determined colorimetrically by matching the colour of lead sulphide obtained from the material with that obtained from standard lead solution.

F-2 REAGENTS

F-2.1 Dilute Nitric Acid, 50 percent.

F-2.2 Concentrated Sulphuric Acid, sp gr 1·84.

F-2.3 Citric Acid, solid.

F-2.4 Ammonia, sp gr 0.880 or diluted as required.

F-2.5 Potassium Cyanide Solution, 10 percent (*m/v*).

F-2.6 Dithizone (Diphenyl Thiocarbazone) Solution, 0·1 percent (*m/v*) solution in chloroform, freshly prepared.

F-2.7 Dilute Hydrochloric Acid, approximately 0.1 N.

F-2.8 Standard Lead Solution

Two reference solutions of lead nitrate are required in this test as given under **F-2.8.1** and **F-2.8.2**.

F-2.8.1 *Standard Strong Lead Solution*, obtained by dissolving 0·096 g of lead nitrate [$Pb(NO_3)_2$] in 50 ml of dilute nitric acid and making up to 100 ml with water.

F-2.8.2 *Standard Dilute Lead Solution*, prepared freshly before the test by diluting 1 ml of standard strong lead solution to 100 ml with water.

F-2.9 Ammonium Acetate, solid.

F-2.10 Sodium Sulphide Solution 10, percent.

F-3 PROCEDURE

F-3.1 Weigh 4·50 to 5·50 g of the material to an accuracy of 0.01 g and add 30 ml of dilute nitric acid in a 100-ml or 200-ml resistance glass or silica Kjeldahl flask. Heat the flask cautiously until there are no more brown fumes, cool and add, very slowly, 10 ml of sulphuric acid. Heat cautiously and continue heating. Evaporate away the sulphuric acid fumes. Dilute with water and cool, digest for some time and heat again to dissolve all the solid. Cool, dilute with about 50 ml of water, add 2 g of citric acid and just neutralize with ammonia. Add one ml of potassium cyanide solution and transfer the whole to a separating funnel. The total volume should be 100 to 150 ml.

F-3.2 Extract the liquid with dithizone solution. Carry out three extractions, using 10·5 and 5 ml respectively; but if the last extraction gives any indication of a reddish tinge, extract again to ensure complete removal of lead.

F-3.3 Take 10 ml of water in another separating funnel and wash each extract with this water. If suspended matter is present in the chloroform extract, it must be filtered before passing to the separating funnel containing 10 ml of wash water. Transfer the combined chloroform extracts to a separating funnel and extract lead by shaking successively with 50, 20 and 10 ml of dilute hydrochloric acid. Combine the acid extracts in a separating funnel, wash once or twice with 10 ml of chloroform and filter through a previously wetted filter paper into a 100-ml graduated flask. Make up the volume of the filtrate to 100 ml with dilute hydrochloric acid and use this as the test solution.

F-3.4 Estimate colorimetrically the lead present by comparison with standard dilute lead solution containing 0.000 006 g of lead per ml (using not more than 10 ml of standard solution for matching) in the following manner:

Transfer a suitable volume of the test solution to a Nessler cylinder. Add 2 g of ammonium acetate, followed by ammonia until just alkaline; then add one ml of potassium cyanide solution. Dilute to 50 ml, add 2 drops of sodium sulphide solution and match the colour against a set of standards prepared in the same way.

F-3.4.1 The limit prescribed in Table 1 for lead shall be taken to be as not having been exceeded if the intensity of the colour produced in the test with the material, is not greater than that produced in the standard lead solution.

F-3.5 A blank determination shall be carried out under the same conditions, on the same reagents and by the same person but without using the material.

ANNEX G
(*Clause* 7.1)
SAMPLING OF FOOD YEAST

G-1 GENERAL REQUIREMENTS OF SAMPLING

G-1.1 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed:

a) Samples shall be taken in a protected place not exposed to damp air, dust or soot.

b) The sampling instrument shall be clean and dry, when used.

c) Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers being sampled from adventitious contamination.

d) Samples shall be placed in clean, free from odour and dry glass containers. The container shall be of such a size that they are almost completely filled by the sample.

e) Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

f) Samples shall be stored in such a manner that the temperature of the material does not vary unduly from normal temperature.

g) Sampling shall be done by a person agreed to between the purchaser and the supplier and in the presence of the purchaser (or his representative) and the supplier (or his representative).

G-2 SCALE OF SAMPLING

G-2.1 Lot

All the containers in a consignment belonging to the same batch of manufacture shall constitute a lot.

G-2.1.1 Samples shall be tested from each lot for ascertaining conformity of the material to the requirements of this specification.

G-2.2 The number of containers to be tested from a lot shall depend on the size of the lot and shall be in accordance with Table 2.

Table 2 Number of Containers to be Selected for Sampling
(*Clauses* G-2.2 *and* G-2 3)

Total Number of Containers in the Lot	Number of Containers to be Selected
N	*n*
(1)	(2)
3 to 50	3
51 to 200	4
201 to 400	5
401 to 650	6
651 and over	7

G-2.3 These containers shall be selected at random from the lot. To ensure the randomness of selection, a random number table as agreed to between the purchaser and the supplier shall be used. In case such a table is not available, the following procedure shall be adopted:

Starting from any container, count them as 1, 2, 3, ... up to *r* and so on in one order where *r* is equal to the integral part of N/n, *N* being the number of containers in the lot and *n* the number of containers to be selected (*see* Table 2). Every *r*th container thus counted shall be withdrawn to give the requisite number of containers in the sample.

G-3 TEST SAMPLES AND REFEREE SAMPLES

G-3 1 Preparation of Individual Samples

Draw, with an appropriate sampling instrument, equal quantities of the material from different parts of the container till about 500 g of the material are obtained. From this, take about 150 g of the material and divide into three equal parts. Each part so obtained shall constitute an individual sample representing the container and shall be transferred immediately to thoroughly clean and dry containers, sealed air-tight and labelled with the particulars given under **G-1.1**(e). The individual samples so obtained shall be divided into three sets in such a way that each set has a sample representing each selected container. One of these sets shall be marked for the purchaser, another for the supplier and the third for the referee.

G-3.2 Preparation of a Composite Sample

From the material from each selected container remaining after the individual sample has been taken, equal quantities of the material shall be taken and mixed together so as to form a composite sample weighing about 600 g. This composite sample shall be divided into three equal parts and transferred to clean and dry containers made of glass and labelled with the particulars given in **G-1.1**(e). One of these composite samples shall be for the purchaser, another for the supplier and the third for the referee.

G-3.3 Preparation of Samples for Microbiological Examination

Draw with an appropriate sampling instrument which is sterile, at least 100 g of the material and mix thoroughly under aseptic conditions to form a sample of that container for microbiological examination. Divide the sample (taking care not to bring in microbiological contamination in the material) into three equal parts. Each part so obtained shall constitute a sample representing the containers and shall be transferred to

sterile glass containers, sealed air-tight and labelled with the particulars given in **G-1.1**(e). They shall be marked, in addition, with the words 'For Microbiological Examination'. The samples so obtained shall be divided into three sets in such a way that each set has a sample representing each selected container. One of these sets shall be marked for the purchaser, another for the supplier and the third for the referee.

G-3.4 Referee Samples

Referee samples shall consist of a set of individual samples (**G-3.1**), one composite sample (**G-3.2**) and a set of samples for microbiological examination (**G-3.3**) marked for this purpose and shall bear the seals of the purchaser and the supplier. These shall be kept at a place agreed to between the two.

G-4 NUMBER OF TESTS

G-4.1 Tests for requirements given under **3.1** and crude protein and free amino nitrogen shall be conducted on each of the samples constituting a set of individual test samples (**G-3.1**).

G-4.2 Tests for moisture, total ash, acid-insoluble ash, thiamine, riboflavin, nicotinic acid, dispersibility, viability, copper and lead shall be conducted on the composite sample as prepared under **G-3.2**.

G-4.3 Test for microflora other than yeast shall be conducted on each of the samples constituting a set of test samples and shall be labelled with the words 'For Microbiological Examination' (*see* **G-3.3**).

G-5 CRITERIA FOR CONFORMITY

G-5.1 The lot shall be considered satisfactory in respect of the requirements tested in **G-4.1** if each of the individual sample satisfies all these requirements.

G-5.2 The lot shall be considered satisfactory in respect of the requirements tested in **G-4.2** if the results of test on the composite sample satisfy the corresponding requirements.

G-5.3 The lot shall be considered satisfactory in respect of the requirement for microflora other than yeast if each of the samples satisfies the specified requirement for microflora other than yeast.

G-5.4 The lot shall be declared to be in conformity with all the requirements of this specification if it has been found satisfactory in **G-5.1**, **G-5.2** and **G-5.3**.

Standard Mark

The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act, 1986* and the Rules and Regulations made thereunder. The Standard Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well defined system of inspection, testing and quality control which is devised and supervised by BIS and operated by the producer. Standard marked products are also continuously checked by BIS for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act, 1986* to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Revision of Indian Standards

Indian Standards are reviewed periodically and revised, when necessary and amendments, if any, are issued from time to time. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition. Comments on this Indian Standard may be sent to BIS giving the following reference:

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